REMARKS

Claims 23-46 are now pending. A "clean" version of the amended claim set is provided above. Indication of the amendments made presently is provided in the section entitled "Version Showing Changes Made", which follows the Remarks. All amendments herein assume that all amendments previously submitted have been made.

Claims 23 and 27 have been amended for clarity. Claim 27 is objected to for recitation of the word "into" with reference to increasing the PEP availability in a pathway. The claim has been amended as suggested by the Examiner.

Rejections under 35 U.S.C. § 112

Claims 23-26, 40, 412 and 43-44 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for recitation of the phrase "host cell comprising a metabolic pathway". Claim 23 has been amended to state that the subject of the claim is a "host cell having a metabolic pathway". The Examiner suggests that since a metabolic pathway is not a component of a cell, the cell cannot comprise the pathway. While not necessarily agreeing with this analysis, Applicants submit that a cell can have a metabolic pathway, the type of pathway being an attribute that a cell can have.

In addition, Claims 43 and 44 are rejected for reciting "The mutant cell of Claim 42", when Claim 42 is actually directed to a method. Claims 43 and 44 have been amended to recite proper antecedent basis.

In light of the above, Applicants submit that the claims satisfy the requirements of 35 U.S.C. § 112, second paragraph. Therefore, withdrawal of this rejection is respectfully requested.

Applicants believe that the previously submitted amendment and remarks address the rejections under 35 U.S.C. §§ 112, first paragraph, 102 and 103.

Applicants submit that the application is in form allowance and early notification of such is earnestly requested. The Examiner is invited to contact the undersigned at (415) 781-1989 if any issues may be resolved in that manner.

Respectfully submitted,

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VERSION SHOWING CHANGES MADE

23. (Thrice Amended) A mutant host cell [comprising] having a metabolic pathway which uses PEP as a precursor or intermediate of metabolism, said host cell characterized by:

(a) being phenotypically Pts-/glu+;
(b) requiring galactose permease activity to transport glucose; and
(c) having a specific growth rate on glucose as a sole carbon source of at least $0.4h^{-1}$.

24. A mutant host cell of Claim 23 comprising recombinant DNA coding for one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.

25. (Once amended) A mutant host cell of Claim 23 further comprising mutations in the pykA and/or pykF genes in said host cell.

26. (Once amended) A mutant host cell of Claim 24 further comprising mutations in the pykA and/or pykF genes in said host cell.

27. (Thrice Amended) A method for increasing PEP availability [into] to a biosynthetic or metabolic pathway of a host cell, the method comprising:

culturing a host cell mutant characterized by:

having a Pts-/glu+ phenotype;
requiring galactose permease activity to transport glucose; and
having a specific growth rate on glucose as a sole carbon source of at least $0.4h^{-1}$;

in the presence of an appropriate carbon source, wherein said host cell mutant utilizes PEP as a precursor or intermediate of metabolism.

28. A method of Claim 27 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of *ptsI, ptsH* and *crr*.

29. A method of Claim 27 further comprising modifying the selected host cell to introduce therein recombinant DNA coding one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.

30. A method of Claim 27 further comprising modifying the selected host cell to reduce or eliminate pyruvate kinase activity in said host cell.

31. A method of Claim 30 wherein pyruvate kinase activity is reduced or eliminated in the host cell by introducing a mutation in DNA encoding one or more of the sequences coding for pyruvate kinase, pyruvate kinase promoter region and other regulatory sequences controlling expression of pyruvate kinase.

33. (Once amended) A method of Claim 42 wherein the DNA used to transform the host cell encodes one or more enzyme(s) selected from the group consisting of DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase, EPSP synthase and chorismate synthase.

34. (Once amended) A method of Claim 42 further comprising transforming the host cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

35. A method of Claim 33 further comprising transforming the host cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

36. (Once amended) A method of Claim 42 wherein the desired compound is selected from the group consisting of tryptophan, tyrosine and phenylalanine.

37. A method of Claim 36 wherein the desired compound is tryptophan and the host cell is transformed with DNA coding one or more gene(s) selected from the group consisting of *aroG, aroA, aroC, aroB, aroL, aroE, trpE, trpD, trpC, trpB, trpA* and *tktA* or *tktB*.

38. (Twice Amended) A method for obtaining a Pts-/glucose+, galactose permease requiring-mutant cell, the method comprising:

(a) selecting a host cell which utilizes a phosphotransferase transport system;

(b) mutating the host cell whereby the phosphotransferase transport system is inactivated;

(c) culturing the mutant host cell using glucose as a carbon source; and

(d) selecting a mutant host cell which grows on glucose at a specific growth rate of at least 0.4 h^{-1}.

39. (once amended) A method of Claim 38 wherein the mutant cells are selected due

to a specific growth rate on glucose of about 0.8 h^{-1}.

40. The mutant cell of Claim 23 having a specific growth rate on glucose as a sole carbon source of about $0.8h^{-1}$.

41. The mutant cell of Claim 23 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

42. A method for enhancing production of a desired compound in a modified host cell, said host cell in its unmodified form being capable of utilizing a phosphotransferase transport system for carbohydrate transport, the method comprising,

(a) culturing a modified host cell with an appropriate carbon source, said modified host cell characterized by having:

(i) a Pts-/glu+ phenotype;

(ii) requiring galactose permease activity to transport glucose;

(iii) a specific growth rate on glucose as a sole carbon source of at least $0.4h^{-1}$; and

(iv) utilizing PEP as a precursor or intermediate of metabolism,

said modified host cell further comprising recombinant DNA encoding one or more enzyme(s) catalyzing reactions in the pathway of biosynthetic production of said desired compound in said modified host cell; and

(b) optionally recovering said compound.

43. (Amended) The <u>method of Claim 42, wherein the modified host cell has</u> [mutant cell of Claim 42 having] a specific growth rate on glucose as a sole carbon source of about $0.8h^{-1}$.

44. (Amended) The [mutant cell] <u>method</u> of Claim 42 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

45. The method of Claim 38 wherein mutating the host cell is by inactivating the phosphotransferase transport system.

46. The method of Claim 45 wherein said inactivating is by deleting part or all of gene(s) selected from the group consisting of ptsI, ptsH and crr.